Exploring combined influences of material topography, stiffness and chemistry on cell behavior at biointerfaces

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SUMMARY

To satisfy the need for a higher quality of life, tissue engineering and regenerative medicine approaches are being developed with the aim to replace or regenerate tissues by combining cells from the body with appropriate biomaterial scaffolds. The design of high-performance biomaterials is vital for the success of biomedical devices and the generation of bio-inspired replacement tissues. Although the physicochemical properties on biomaterial surfaces were well-demonstrated to significantly affect (stem) cell fate, mastering the complexity of biomaterials and its interaction with cells are still in its infancy due to a lack of highly efficient experimental platforms. In this thesis, advanced material interfaces have been developed to explore and elicit their interactions with (stem) cells for improving and accelerating the development of high-performance biomaterials.

Chapter 1 provides a general introduction on the native cellular microenvironment, particularly the ECM, and describes how cells interact with physicochemical properties of functional and biomimetic materials, as well as their current challenges, which leads to the objectives of this thesis.

In order to explore how multi-parameter material interfaces influence cell response, aligned nanotopographical surfaces with different mechanical properties were developed and applied for a deeper understanding of cell-material interactions in **Chapter 2**. PDMS wrinkled structure as the template was translated to pHEMA hydrogels via hydrogel imprinting lithography, resulting in the same topography feature and dimension but with a different stiffness. To the best of our knowledge, for the first time nanotopographical effects were combined with material mechanical properties differing over two orders of magnitude in Young's modulus. The biointerfaces were used to evaluate osteoblast-like cells (SaOs), fibroblasts and lens epithelial cells (LECs) from tissues with different intrinsic stiffness. Differing from our initial anticipation, LEC adhesion was inhibited by the soft topography while SaOs adhesion was limited by the stiff structure. However, both collagen I production and ALP expression were more stimulated on the hard interfaces after 5 d culture, particularly on the hard topographical surface. The soft structure caused fibrosis formation due to upregulation of collagen I and α-SMA for HSkF and LEC. Therefore, the presented platforms provide a new strategy for studying how complex biointerfaces interact with cells, which will facilitate biomedical materials design more rapidly and accurately.

In addition, gold nanowire-patterned array platforms with the multi-scale design from the macroscale to the nanoscale were developed for investigating human bone marrow-derived mesenchymal stem cell (hBM-MSC) response in **Chapter 3**. Facile control over the biomimetic Au nanowire structures on the glass substrate can affect cell organization through the synergistic effects of surface micro/nanotopography and chemical cues. The anisotropic nanobiointerface can result in the stabilization and oriented growth of focal adhesions and sequentially generate contractile and aligned cytoskeleton actin. Interestingly, when the angle of the Au nanowires on the glass is increased from 0° to 90°, hBM-MSC arrangement exhibits a transition from a unidirectional distribution induced by a vector response to a bimodal polarization pattern. The degree of cell vector response and elongation decreased with increasing Au nanowire angles from 0 to 90°. Further, we demonstrated that the specific cell adhesion and organization are dependent on the surface micro/nanotopography, which is greatly enhanced by introducing stem cell-material affinity differences due to the difference in protein adsorption amount and initial rate between glass and gold. The presented

nanobiointerface will also offer a new methodological platform to explore neural tissue behavior or intracellular delivery of therapeutic macromolecules under electrical or thermal stimulation. Moreover, the surface chemistry on this platform can easily be modified to incorporate functional groups or bioactive factors (protein), increasing the biointerface complexity required to study cell-surface interactions further.

Biomaterial surface properties offer critical cues to direct (stem) cell behavior and not always in a predictive fashion presented in **Chapter 2&3**. In order to better understand the relationship between biomaterial properties and biological performance as well as identify the optimal cellular response, high-efficient and complex bioanalysis platforms were designed in **Chapter 4-6**. Surface gradients provide a powerful HTS platform to accelerate multiscale design and optimization of material properties to enhance the function of biomaterials. **In Chapter 4**, surface-aligned nanotopography gradients based on PDMS were prepared via strain-oxidation-release procedure with different plasma treating times for studying osteoblast-like cell adhesion and alignment more efficiently and accurately. It was found that the osteoblast-like cell behavior is influenced by small changes in surface topography. Less focal adhesions with increasing wrinkle dimensions (i.e., amplitude, and wavelength), while focal adhesion orientation and cell alignment initially increased with increasing wrinkle features and subsequently decreased again. Importantly, it was identified that wrinkles with 130-180 nm amplitude and 550-730 nm wavelength are the most favorable topography for inducing the cell alignment while amplitudes of 15-45 nm and wavelengths of 400-520 nm are the optimal combined parameter response to regulate cell behavior. The cellular focal adhesion results on the gradient surfaces disproved a linear relationship of cell adhesion towards aspect ratio as was found on uniform substrates.

Using above gradients, a novel strategy was developed to translate PDMS-based wrinkled gradients to various clinically relevant inorganic biomaterials for studying hBM-MSCs responses *in vitro* in **Chapter 5**. We found a positive correlation between cell alignment and the orientation of cytoskeleton, filopodia, and focal adhesions. The optimal wrinkle feature (wavelength: 7121 nm; amplitude: 2561 nm) for promoting hBM-MSCs alignment, cytoskeleton arrangement, long/parallel filopodia as well as focal adhesion assembly and orientation was identified based on above platforms. The topography and interface material on the gradient platforms have combined effects on the response of hBM-MSCs, which indicates that we need to apply a screening to assess optimum conditions for both current and new biomaterials.

In order to improve efficiency and increase complexity, in **Chapter 6** an enhanced HTS platform system combined with the orthogonal double gradient surface was developed to elucidate combined physical parameter influences on stem cell behavior and thereby gain insights in hBM-MSC responses. Surface stiffness and wettability on the gradient vary independently and continuously in perpendicular directions within a single sample. Each location on the surface has a unique parameter combination over a broad range prepared by plasma oxidation and chemical modification. It was found that most cell responses are non-linearly regulated by material stiffness and wettability. The optimal combined surface properties for promoting hBM-MSCs adhesion, nucleus size, spreading as well as vinculin expression were obtained based on the orthogonal double gradient platform. The platform allows for efficient analysis of the relationship between biomaterial properties and biological performance to develop biomaterial libraries,

which could accelerate the creation of next generation biomaterials for biomedical engineering.

In the general discussion (**Chapter 7**), the major findings of this thesis and possibilities for future research in this field were highlighted. It displays that complex material interfaces were designed and developed to explore cell-material interactions and elicit the relationship between biomaterial properties and biological performance to be used in the future as possible advanced tissue engineering and regenerative medicine approaches. Cells always integrate multiple cues from their microenvironment and we should include as many different parameters in our biomaterials as possible and study these for an accurate description of the cell state as a consequence of interacting with a material. In order to highly control cellular behavior, it is crucial to identify the optimal cell response by studying a detailed interaction between cells and materials over a broad range. The outcomes in this thesis are expected to act as a catalyst for other researchers to efficiently explore cell behavior from a more complex point of view. Our work is not just to obtain more knowledge on cell and material interactions, but to apply this knowledge for accelerating the development of high-performance biomaterials, which can become commercially available.